Profile Changes of Putative Periodontal Pathogens after Non-surgical Periodontal Treatment

R.Y.L. MAK*, K.Y. ZEE, W.K. LEUNG and E.F. CORBET (Faculty of Dentistry, The University of Hong Kong)

BACKGROUND:

- Bacteria are shown to be the etiological agents of periodontal disease (Haffajee and Socransky 1994).
- A number of microbial species like *A. actinomycetemcomitans, B. forsythus and P. gingivalis* are implicated as the putative pathogens associated with adult periodontitis (Consensus report, AAP, 1996).
- Scaling and root planing is consistently shown to be effective initial non-surgical periodontal therapy (Cobb 1996).
- After the introduction of Polymerase Chain Reaction (PCR) identification method of oral putative pathogens by Ashimoto et al. 1996, no study has made use of their protocol in monitoring longitudinal change of bacterial profile after non-surgical periodontal therapy.

OBJECTIVES:

- To describe the profile of the 8 putative periodontal pathogens using PCR in patients with moderate to advance periodontal disease.
- To describe the changes of the profile of the pathogens after non-surgical periodontal treatment at 1 month.
- To describe the changes of clinical parameters before and at 3 months after non-surgical periodontal treatment.
- To correlate the qualitative changes of the periodontal pathogens, if any, to the changes of the clinical parameters observed.

MATERIALS AND METHODS:

Subjects:

4 male and 10 female patients (mean age 43.7) with moderate to severe periodontitis.

Full mouth clinical data :

- Supragingival plaque (Pl%)
- Probing pocket depth (PPD)
- Probing attachment level (PAL)
- Bleeding on probing (BOP%)
- at six sites per tooth except third molars
- using a custom-made soft acrylic occlusal stent as a reference guide

Calibration:

The weighed kappa values for repeated PPD and PAL measurement were 0.78 and 0.81 respectively and reached a reproducibility of 95% within 1mm deviation.

Microbial samples:

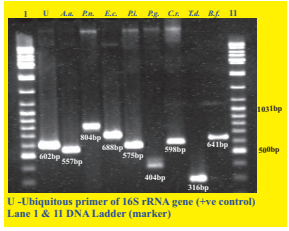
Microbial samples were taken from the deepest sites of two separate teeth of each quadrant by sterilized paper points after supragingival plaque removal using sterile cotton pellets.

Microbial identification:

According to a protocol modified from Ashimoto et al. (1996), microbial samples were processed with a 16S rRNA-based polymerase chain reaction (PCR).

Actinobacillus actinomycetemcomitans (A.a.)
Bacteroides forsythus (B.f.)
Campylobacter rectus (C.r.)
Eikenella corrodens (E.c.)
Porphyromonas gingivalis (P.g.)
Prevotella intermedia (P.i.)
Prevotella nigrescens (P.n.)
Treponema denticola (T.d.)

were identified by running the PCR products through 1.5% Agarose Gel Electrophoresis as in the photograph above.



U -Ubiquitous primer of 16S rRNA gene (+ve control)
Lane 1 & 11 DNA Ladder (marker)

Table 1

Clinical parameters

	Baseline	3-month
PL%	84%	34% *
BOP%	92%	36% *
Mean PPD	3.69mm	1.91mm*
PPD reduction		
Sites<4mm		0.95mm* ±0.34
Sites 4-6mm		2.48mm* ±0.35
Sites>6mm		4.26mm* ±0.86
PAL gain		
Sites<4mm		0.22mm** ±0.24
Sites 4-6mm		1.24mm* ±0.34
Sites>6mm		2.14mm* ±0.66

* $p<0.001$ ** $p<0.005$ *t*-test

RESULTS:

Clinical parameters:

Table 1 shows the changes in clinical parameters 3 months after non-surgical periodontal therapy.

Microbial parameters:

At subject level, Figure 1 shows the changes in detection frequency of the 8 putative pathogens at baseline and 1-month after therapy.

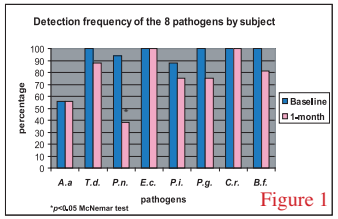


Figure 1

At site level, Figure 2 shows the changes in detection frequency of the 8 patative pathogens at baseline and 1-month after therapy.

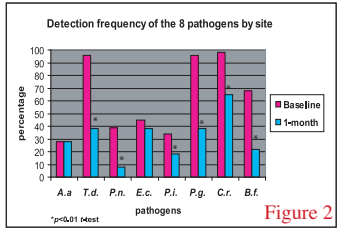


Figure 2

Microbial association:

Significant association ($p<0.001$ Fisher's Exact Test) was found in 3 pairs of the pathogens namely *T.d./P.g.*, *T.d./C.r.* and *P.g./C.r.* which yield odds ratios of 30.7, 43.7 and 38.5 respectively. The pattern of association could be illustrated in Figure 3 showing the possible co-occurrence of these 3 species.

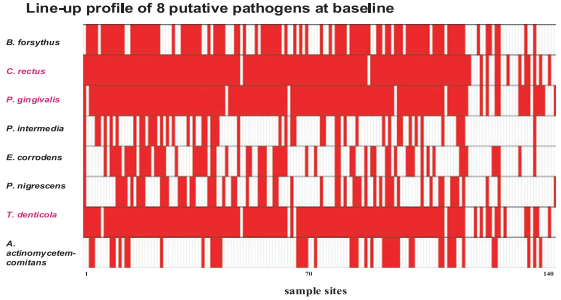


Figure 3

Microbial and Clinical Outcome:

The presence of the microbial complex (*C.r./P.g./T.d.*) was significantly associated with deeper sample site PPD at baseline, 6.8mm *vs*. 5.4mm when not all three were present.

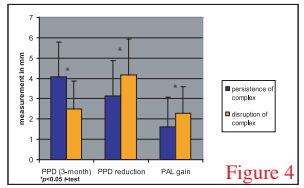


Figure 4

Comparing sites with persistence of the complex to those at which the complex had been disrupted at 1 month after treatment, deeper residual PPD (4.1mm *vs*. 2.5mm), less PPD reduction (3.1mm *vs*. 4.2mm) and less PAL gain (1.6mm *vs*. 2.3mm) were found at the 3-month examination. (Figure 4)

CONCLUSIONS:

- The 3-month response to non-surgical therapy in this group of moderate to severe chronic periodontitis patients is comparable to other similar studies.
- 7 out of the 8 putative pathogens are frequently detected in the subgingival plaque of this group of patients.
- Three species namely *T. denticola, P. gingivalis* and *C. rectus* are found occurring in close association in the subgingival plaque.
- The presence of the complex at a site is associated with deeper baseline probing pocket depth.
- The persistence of the complex at 1-month is associated with deeper residual PPD, less PPD reduction and less gain in PAL at 3-month.

References:

Ashimoto et al. (1996) *Oral Microbiol. Immunol.* **11**:266-273.
Cobb (1996) *Ann. of Periodontol.* **1**:443-490.
Consensus report (1996) *Ann. of Periodontol.* **1**:926-932.
Socransky & Haffajee (1994) *Periodontol. 2000* **5**:7-25.

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